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**Lactate transporters as therapeutic targets in cancer and inflammatory disease**

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## Abstract

**Introduction:** Inflammation is associated with the accumulation of lactate at sites of tumour growth and inflammation. Lactate initiates tissue responses contributing to disease. We discuss the potential of targeting lactate transporters in the treatment of cancer and inflammatory conditions.

**Areas covered:** Lactate is the product of glycolysis, which is considered to be a waste metabolite and a fuel for oxidative cells. It is also an active signalling molecule with immunomodulatory and angiogenic properties. They are the consequence of lactate binding to membrane receptor(s) or being transported through specific carrier-mediated transporters across the cellular membrane. Carriers are distinct in proton-linked monocarboxylate transporters (MCTs) and Na<sup>+</sup>-coupled electrogenic transporters (SMTCs), expressed by several tissues including immune system, endothelium and epithelium. Several tumours and inflammatory sites (i.e., arthritic synovium, atherosclerotic plaque) show accumulation of lactate and altered expression of its transporters, thus suggesting a role of this metabolite in cancer and inflammatory disorders. We review the most recent evidence on lactate biology, focusing on transporter expression and function in health and disease.

**Expert opinion:** Lactate-initiated signalling is gaining attention for its implications in cancer and inflammation. This review deals with the therapeutic potential of targeting lactate transporters and drugs that are already in clinical use for cancer and discusses the opportunity to develop new therapeutics for inflammatory disease based on recent findings.

#### **Article Highlights:**

1. Lactate is a main carbon source for biosynthetic pathways
2. Lactate is an immunomodulatory metabolite
3. Lactate transporters are expressed at different levels in immune, endothelial, epithelial and cancer cells
4. Expression of lactate transporters is up-regulated in some cancers and inflammatory disorders
5. Targeting lactate transporters is a therapeutic avenue that may lead to reduced inflammation and promote anti-cancer immunotherapy

## 1. Introduction - Lactate biology

Lactate is produced from pyruvate by the enzyme lactate dehydrogenase (LDH) in an NADH-dependent reaction. Its production occurs mainly in the cytoplasm under hypoxic conditions (fermentation) or even in the presence of normal oxygen concentration as a consequence of high glycolytic activity, such as in proliferating cells (we refer to this phenomenon as aerobic glycolysis, or Warburg effect, from the name of the scientist who first described it in cancer cells (1)). The production of lactate is important to replenish the pool of NAD<sup>+</sup> necessary to maintain the flux of glycolysis. Lactate can be oxidised back to pyruvate and fed in to the TCA cycle or used as a fuel source for gluconeogenesis in the liver (2).

Lactate is present in solution in two distinct forms: as acid (i.e. lactic acid) at low pH, or as an ion salt (i.e. sodium lactate) at higher pH, with a dissociation constant (pK<sub>a</sub>) of 3.86. Lactate transfer across the plasma membrane happens through specific solute carrier transporters that we describe in more details in a separate section of this review. The physiological concentration of lactate, in blood and healthy tissues, is about 1.5-3mM (3), but in pathological conditions, such as atherosclerosis, rheumatoid arthritis and cancer, can peak at 10-30mM (4-6).

For many years lactate has been regarded merely as a waste product. Nowadays, emerging evidence has rehabilitated it to a major carbon source fuelling metabolic pathways as well as a proper signalling molecule able to orchestrate a variety of biological processes.

Many recent findings come from the field of cancer biology, because of the observation that in the cancer microenvironment lactate concentration can rise up to 20-30 mM (4, 5). Lactate is able to promote migration and growth of cancer cells (7, 8) and, in some cancerous cells, is also utilized as a substrate for the synthesis of lipids (9).

In a paper published in *Cell* in 2017, Faubert et al. investigated the fate of lactate in human lung cancer. Using intra-operative infusions of <sup>13</sup>C-labeled nutrients, they were able to analyse the pattern of enrichment of labelled metabolites directly within the tumour (in this case lung tumours). The infusion of [U-<sup>13</sup>C] glucose showed a different pattern of enrichment in the cancer tissue as compared to the benign lung, with an excess of labelled lactate (rather than other upstream glycolytic metabolites) within the tumour. Interestingly, the infusion of <sup>13</sup>C-lactate caused not only the accumulation of labelled lactate itself in the tumour, but also of labelled pyruvate, alanine and TCA cycle intermediates, showing that lactate was actively being used to supply the TCA cycle. The authors confirmed these findings in xenograft mouse models, but we believe the most important conclusion of the research was that comparing the contributions of lactate and glucose to the TCA cycle in the grafted tumours, the former turned out to make a much larger contribution than the latter. This effect was more dramatic when tumours were grown orthotopically in the lung rather than subcutaneously, suggesting a specific contribution of the tissue of residence of the tumour. Importantly, MCT1-deficient tumours showed a lower labelling of lactate-derived metabolites, underlining the importance of this lactate transporter (10).

Later in 2017, Hui et al. published another important piece of research in *Nature* demonstrating the role of lactate in fuelling the TCA cycle, both in normal and cancerous tissues. Analysis of the flux of circulating metabolites in mice showed that lactate is a major source of carbon for the TCA cycle; the infusion of <sup>13</sup>C-lactate, in fed and fasted mice, showed extensive labelling of TCA cycle metabolites in all tissues, and in lung and pancreatic tumours the contribution of circulating lactate to the activity of TCA cycle was greater than that of glucose. These findings demonstrate that lactate is a substrate

of the TCA cycle both in normal and cancerous tissues, as well as that glycolysis and TCA cycle are uncoupled at the level of lactate, thus allowing the independent regulation of the two processes which also happen in a tissue-specific manner (11).

The effects of lactate are not only due to its ability to feed metabolic pathways, but also to its ability to trigger a signalling pathway via its receptor Gpr81. This is a surface lactate receptor involved in the regulation of lipolysis (12) but also in cancer cell survival (13). Feng et al., recently showed that lactate, through Gpr81, is able to regulate the expression of PD-L1 in human lung cancer cells. PD-L1 is the ligand of PD1, a receptor expressed on the membrane of activated T cells, responsible for reduced proliferation and effector function of T cells, and a major target for cancer immunotherapy (14). The authors show that lactate upregulates the expression of PD-L1 at a transcriptional level, in a Gpr81-dependent manner, and this upregulation leads to suppression of the effector function of T cells in co-culture experiments. Overall, the data point to a role for lactate in the tumour escape from immune-surveillance (15).

The ability of lactate to interfere with the response of the immune system against tumours was previously reported by the Kreutz' group. In 2007 they showed how tumour derived-lactic acid inhibits the proliferation and cytokine production of cytotoxic CD8<sup>+</sup> T cells, while also dampening their cytolytic activity (16). The same group later showed that lactic acid also inhibits the production of TNF by human monocytes and decreases their glycolytic activity (17). More recently they published in *Cell Metabolism* a comprehensive analysis of the effects of lactic acid on immune surveillance against cancer (18). The authors generated melanoma cell lines with reduced expression of *Ldha* and observed a reduced rate of tumour growth as compared to controls. Upon analysis of the immune infiltrate, they found *Ldha*-low tumours bearing more immune cells, particularly CD8<sup>+</sup> T cells producing IFN $\gamma$  and granzyme B. This increase in the activity of CD8<sup>+</sup> T cells was due to reduced production of lactic acid. Indeed the authors showed that lactic acid impairs both function and survival of T and NK cells. They went on to address a mechanism behind this phenotype. The lactic



acid produced by the tumour was taken up by T cells, causing a decrease in their intracellular pH that led to a drop in ATP production. This caused an impairment of the T cell functions, as a consequence of reduced NFAT activity, which is involved in the transcriptional control of IFN $\gamma$  (18).

Along similar lines, the work published by Colegio et al. in 2014 showed that the lactic acid produced by tumours (lung carcinoma and melanoma) is able to induce the expression of Vegf and Arg1 in tumour-associated macrophages (TAMs), in a HIF1 $\alpha$ -dependent manner. Moreover, lactic acid was also able to polarize TAMs toward a M2-like phenotype, thus enabling the establishment of a permissive microenvironment for tumour growth (5).

In the context of the tumour microenvironment, lactate plays a role not just in the crosstalk between cancer and immune cells, but also between cancer cells on one side and endothelium and stromal fibroblasts on the other side. Vegran and co-workers demonstrated that in the tumour microenvironment the lactate produced in large part by the cancer cells is taken up by endothelial cells (EC) leading to NF $\kappa$ B activation and consequent production of IL8, which in turn drives EC migration and neo-angiogenesis supporting tumour growth (19). Similarly and in agreement with (5), Sonveaux et al. showed that lactate is also able to activate HIF1 in EC, increasing the production of pro-angiogenic factors (bFGF and VEGFR2), thus overall stimulating angiogenesis; the inhibition of MCT1 is sufficient to abrogate the pro-angiogenic effect of lactate (20). Lactate is also important in the interplay between cancer and stromal cells. Fiaschi et al. have identified a lactate shuttle between human prostate cancer cells and cancer associated fibroblasts (CAF): the physical interaction between cancer cells and fibroblasts leads to the differentiation of the latter to CAF; these cells show high glycolytic activity with generation of lactate, elevated expression of MCT4 and stabilisation of HIF1. The lactate produced and released by CAF is then taken up by cancer cells through the transporter MCT1 and it is metabolised in the mitochondria, sustaining cancer growth. The disruption of this shuttle via pharmacological inhibition or genetic silencing of MCT1 inhibits cancer proliferation both in vitro and in vivo (21).

Diverse subsets of T cells show different metabolic requirement, with cytotoxic and effector T cells relying more on glycolysis for proliferation and cytokines production (22, 23) and, conversely, regulatory T cells (Tregs) being more dependent on oxidative phosphorylation (OXPHOS) (24, 25). Recently, Angelin et al. demonstrated that Foxp3 is able to reprogram the metabolism of Tregs, allowing them to cope better in low-glucose, high-lactate microenvironments. They found that the transcription factor Foxp3 promotes the increase of the oxygen consumption rate in inducible Tregs (iTregs) and also the production of reactive oxygen species (ROS); moreover Foxp3 is able to suppress the activity of Myc by binding to its promoter and reducing the expression of Myc-dependent transcripts, most of which are involved in the regulation of glycolysis. Foxp3 was also found to be able to regulate the direction of the LDH reaction in favour of the oxidation of L-lactate to pyruvate, leading to decreased production of lactate by Tregs (as compared to conventional T cells). Tregs can sustain exposure to lactate with better efficiency than effector and cytotoxic T cells, which are instead impaired by it: effector cells require NAD<sup>+</sup> replenishment to sustain the flux of glycolysis, but the excess of utilization of lactate by LDH depletes the available pool of NAD<sup>+</sup>, leading to a decreased glycolytic flux; Tregs instead possess higher levels of NAD<sup>+</sup> and due to their intrinsic metabolism are less affected by reduced glycolysis. The authors discuss that this adaptation could be detrimental in the tumour microenvironment, where the high concentration of lactate may inhibit anti-tumour immunity without affecting the regulatory component, which can further dampen the immune response against the tumour (26).

Recent evidence shows that lactate plays a crucial role also in the orchestration of the immune response in inflammatory conditions. In 2016, Peng et al. formally demonstrated that LDHA activity is necessary in CD4<sup>+</sup> T cells to sustain aerobic glycolysis and express interferon- $\gamma$  (IFN- $\gamma$ ), thus allowing a proper differentiation to the T helper 1 (Th1) subset. The authors found that genetic deletion of LDHA in CD4<sup>+</sup> T cells reduced significantly glucose consumption, promoting a shift towards an oxidative metabolism, and, more importantly, led to a reduction of IFN- $\gamma$  expression. The reduction of IFN- $\gamma$  transcripts (along with many others), was due to an overall decrease of histone

acetylation. Histone acetylation requires acetyl-coenzyme A (acetyl-CoA), but in the absence of LDHA, the consequent increased flux of the TCA cycle does not allow the export of acetyl-CoA from the mitochondria to the cytosol, reducing the pool of acetyl groups. These data demonstrate that LDHA regulates INF- $\gamma$  production in Th1 cells through a fine-tuned epigenetic mechanism of histone acetylation coupled with the cellular metabolism (27).

Lactate is also involved in the production of IL17 and differentiation to the Th17 subset. In 2011, Yabu et al. showed how lactic acid enhances the production of IL23/IL17 by CD4<sup>+</sup> T cells, acting as a pro-inflammatory signal (28). More recently, Haas et al. showed that sodium lactate is able to modulate T cell effector function by promoting the up-regulation of IL17 expression. They also showed that lactate affects the migratory capabilities of CD4<sup>+</sup> T cells, thus causing their retention in the site of inflammation. These findings have an important impact on the understanding of the role of lactate in the inflammatory site, such as the inflamed synovium in rheumatoid arthritis, where lactate may act as an inflammatory signal leading to entrapment of CD4<sup>+</sup> T cells and stimulation of IL17, thus perpetrating the inflammatory process. Interestingly, all these effects are mediated via a specific sodium lactate transporter, SLC5A12, which is expressed on the membrane of CD4<sup>+</sup> T cells (6).

Overall lactate may induce an immune suppressive response in cancer yet act as an inflammatory signal in inflammatory conditions. The different response may be due to the context-dependent availability of nutrients and competition for them by the cellular constituents of the microenvironment (further discussed in Expert Opinion Section).

Lactate is also important in the physiology of the brain, through the astrocyte-neuron lactate shuttle (ANLS). This lactate exchange was first described in 1994 and highlights the existence of lactate-producing cells (astrocytes) and lactate-consuming cells (neurons): in this model, the neurotransmitter glutamate released in the synapse triggers glucose uptake and therefore lactate production by astrocytes; lactate is then utilised by neurons as a source of energy (29). For a more

detailed description of the role of lactate in the brain, both as a metabolite and a signalling molecule, we point the reader to a recent review (30).

Taken together, it is clear that lactate is not just an end-product of metabolism, but rather a source of carbons and, more importantly, a signal that affects the behaviour and the differentiation of many different cell types, especially at the interface between cancer and immune cells and in inflammatory diseases. In the following sections of this review we focus on the role of the different lactate transporters for their biological roles and as putative therapeutic targets.

## **2. Lactate transporters**

### **2.1 Monocarboxylate transporters (MCTs)**

Monocarboxylate transporters (MCTs) include 14 transmembrane proteins encoded by the SLC16A gene family. According to the Milton Saier classification (<http://www.tcdb.org>), MCTs belong to the monocarboxylate porter (MCP) family, which in turn is part of the facilitator superfamily (MFS). MCTs have been identified in all eukaryotic organisms and can transport a wide variety of substrates (31) (Table 1).

MCT1-4 are proton-linked transporters responsible for transport across the plasma membrane of several monocarboxylate metabolites, such as pyruvate, L-lactate and ketone bodies (acetoacetate and D- $\beta$ -hydroxybutyrate) together with a proton (32, 33). Other identified MCTs are MCT8 which shows high affinity for the thyroid hormones T3 and T4, and MCT10/TAT1, a transporter of aromatic amino acids (33, 34). MCT6 has been reported to facilitate the proton-linked transport of bumetanide (35). MCT7 has been implicated in the export of ketone bodies by hepatocytes (36).

MCT9 has been identified as a sodium- and pH-independent carnitine efflux transporter upon expression in *Xenopus* oocytes injected with [3H]-carnitine (37). The substrates and functions of the other MCT family members are yet not known.

MCTs are expressed in a wide range of tissues (such as brain, skeletal muscle, heart, bowel and liver) and display many physiological functions. In particular they play a pivotal role in the control of the central metabolism of glucose, gluconeogenesis, and activation of T-lymphocytes, spermatogenesis, pancreatic  $\beta$  cell activity, thyroid hormone metabolism and drug transport (31).

Among other functions, MCTs are important regulators of intracellular lactate and pH. Indeed, highly glycolytic cells, such as inflammatory and tumour cells, utilize MCT transporters to export lactate.

Lactate is one of the main substrates of MCT1–4. This metabolite is generated from pyruvate (produced from glycolysis and glutaminolysis) during lactic fermentation. In most normal tissues where lactate is produced, MCT1 (SLC16A1) is responsible for its export across the plasma membrane in to the extracellular space (33, 38).

Lactate can be taken up from the extracellular space and used as a substrate to fuel metabolic pathways such as lipogenesis, gluconeogenesis, TCA cycle and oxidative phosphorylation (OXPHOS) (28, 10). Cells that utilize lactate may express different MCTs depending on tissues and species (33, 39-42).

In many cancer cells with an oxidative metabolic fingerprint, MCT1 is the most expressed MCT isoform (40, 43). However, in glycolytic cancer cells and other specific tissues such as white muscle fibres and astrocytes, MCT4 is expressed at higher level than MCT1 and mediates lactate export (40, 43, 45). Accordingly, there is increasing evidence in support of the shuttling of this metabolite between cells with different metabolic behaviours within the same tissue. Such phenomenon has been described in the skeletal muscle where glycolytic/white fibres export lactate through MCT4 and oxidative/red fibres import lactate through MCT1 to fuel the TCA cycle (46). A similar mechanism has been proposed to account for a metabolic symbiosis between glycolytic/hypoxic cancer cells and oxidative/oxygenated cancer cells in tumours (40). Notoriously in the brain, glycolytic

oligodendrocytes and astrocytes export lactate through MCT1 and MCT4 to fuel oxidative neurons expressing MCT2 (47-50).

## 2.2 Sodium-coupled transporter (SMCTs)

In contrast to MCTs, which function as H<sup>+</sup>-coupled electroneutral transporters, SMCTs function as Na<sup>+</sup>-coupled electrogenic transporter. The transport process is electrogenic as more than 1 Na<sup>+</sup> is transported per transport cycle with a Na<sup>+</sup>/monocarboxylate substrate ratio of  $\geq 2$ . Two members of the sodium-coupled monocarboxylate transporter family (SMCT) have been identified so far, the high-affinity transporter SMCT1 (SLC5A8) and the low-affinity SMCT2 (SLC5A12) (51, 3). The SMCT1/SLC5A8 gene was originally identified from a library of kidney cDNA as a close structural relative of the human Na/I symporter (SLC5A5) (51). Other than in the kidney, SLC5A8 has been subsequently detected in intestine, salivary gland, thyroid gland, brain, and retina (52). Substrates of SLC5A8 are similar to those of MCTs (53, 54). SLC5A8 mediates the transport of monocarboxylic acids such as lactate, pyruvate, propionate, butyrate, nicotinate, and short-chain fatty acids (Table 2).

The affinity of the transporter for these monocarboxylates is quite high, with a Michaelis constant in the range of 200–400  $\mu$ M.

Less is known about SMCT2/SLC5A12. mRNA expression of SLC5A12 was detected in kidney, small intestine, and skeletal muscle and to a lesser level in brain and retina. Functional characterization of SLC5A12 suggested a substrate specificity similar to that of SLC5A8. However, the affinities of SLC5A12 for monocarboxylate substrates are approximately 35- to 80-fold lower than those of SLC5A8 (3).

In the kidney, SLC5A8 is expressed in the apical membrane of tubular epithelial cells in the S2-S3 proximal tubule segments. Here SLC5A8 is involved in renal reabsorption of lactate and pyruvate in a

sodium-dependent transport (55-58). Indeed, SLC5A8-deficient or knockout mice exhibit increased urinary excretion of lactate (59). Renal SLC5A12 is localized at the brush border with higher expression in the initial part of the proximal tubules and gradually decreasing toward the S3 segment. Thus, the proximal convoluted tubules provide low and high affinity transporters in the upper and lower proximal tubules, respectively.

In the brain, SLC5A8 exhibits a neuron-specific distribution and may mediate cellular uptake of lactate and ketone bodies, the primary energy substrates of neurons (60), while SLC5A12 is specifically expressed by astrocytes. Besides its physiological functions, several reports suggested a tumour suppressing role for SLC5A8. High frequency of aberrant methylation or down-regulation of the SLC5A8 gene has been observed in human colon cancer, papillary thyroid carcinomas, pancreatic cancer, prostate tumour, acute myeloid leukemia, and glioma formation (52, 61, 62). Interestingly, Li et al. found that the exogenous SLC5A8 was able to suppress the proliferation of colon cells carrying the defective allele, thus suggesting that SLC5A8 inactivation confers a selective advantage to neoplastic colon epithelial cells (62). The mechanisms of this effect remain to be explored.

In the bowel, SLC5A8 is expressed differentially in the lumen-facing apical membrane of colonic and intestinal epithelial cells, while SLC5A12 is expressed primarily in the small intestinal tract (63, 53).

Recently these transporters have been also linked with the function of immune cells. Interestingly, SLC5A12 has been identified to be expressed by human and murine lymphocytes. In particular it was found that SLC5A12 is selectively expressed on the surface of CD4<sup>+</sup> but not of CD8<sup>+</sup> T cells (6, 64, 65). Whether immune cells express SLC5A8 is yet not known.

### **3. MCTs in cancer and immunity**

MCTs, especially MCT1 and MCT4, are widely expressed in a variety of immune and cancer cells. In the tumour microenvironment, cancer cells produce high amount of lactate, which is extruded in the intercellular space via MCT4. Released lactate is then taken up by macrophages via

MCT1, promoting their polarization toward a TAM phenotype via the induction of HIF1 $\alpha$  and arginase 2. These effects induce neo-angiogenesis, via vascular endothelial growth factor (VEGF) production, and ultimately promote tumour growth (5).

MCT1 expression has been reported in a variety of human malignancies including head and neck, lung, stomach, colon, prostate and cervix, as well as gliomas (40, 66-68). MCT1 has also been proposed to be the most important isoform responsible for lactate transport across the plasma membrane in breast and bladder cancer, non-small cell lung carcinomas (NSCLC) and ovarian carcinomas (69,70).

MCT4 is also widely distributed in different cancer types. Its expression has indeed been reported in breast, colon, bladder, and prostate cancers, as well as in cancers of the gynecologic tract and gliomas (66-69).

Recently, Pertega-Gomes and Baltazar (71) reported a correlation between the expression of MCT1, MCT2 and MCT4 and the different stages of prostate cancer progression. MCT1 and MCT2 play a role in tumour maintenance, whereas MCT4 increases tumour aggressiveness. MCT2 was also proposed as a biomarker for prostate cancer (71). In another study focused at NSCLC, Eilertsen et al. proposed MCT1 as a biomarker for prognostic and survival (70). The co-expression of GLUT1 and MCT1 and of GLUT1 and MCT4 was found to be a negative prognostic factor associated with poor disease-specific survival.

MCT2 and MCT4 show a high intracellular expression. This suggests a possible role of these transporters in mediating lactate and/or pyruvate transport across the membranes of intracellular vesicles or organelles (45, 69).

Lactate transporters have been described in inflammatory sites such as rheumatoid arthritis (RA) synovium (6, 65). Here lactate is produced by highly glycolytic local cells such as fibroblast-like synovial cells as well as infiltrating immune cells (i.e. lymphocytes, and macrophages).

Unlike in tumours where lactate plays a key role in promoting cancer cell migration and growth, in inflammatory sites lactate activates a stop migration signal leading to the local entrapment of T cells



(72, 6). This phenomenon is due to the interaction of sodium lactate and lactic acid with the transporters SLC5A12 and SLC16A1/MCT1 which are selectively expressed on the surface of CD4<sup>+</sup> and CD8<sup>+</sup> subsets, respectively (6) (Figure 1). Sodium lactate-mediated inhibition of CD4<sup>+</sup> T cell migration is regulated via lactate interference with metabolic pathways (64, 72). Sodium lactate via SLC5A12 prompts CD4<sup>+</sup> T cells to start producing higher amounts of pro-inflammatory cytokines, in particular IL17A, while lactic acid causes impairment of CD8<sup>+</sup> T cell-mediated killing through SLC16A1/MCT1 (6, 16, 73). Lactate also promotes T cell retention in the inflamed tissue and exacerbates the process of chronic inflammation (i.e. synovial tissue) (6) (Figure 1). Similar to cancer cells, highly proliferating RA synovial fibroblasts (RASFs) express high levels of MCT4, thus promoting synovial fluid acidification (74) (Figure 1). Silencing of MCT4 led to inhibited proliferation of RASFs and reduced the severity of arthritis in the mouse model of collagen-induced arthritis (CIA) (74). Accordingly, other authors found that MCT4 is required for macrophage activation upon TLR2 and TLR4 stimulation. MCT4 knockdown led to enhanced intracellular accumulation of lactate and decreased glycolysis in LPS-treated macrophages reducing their active response during inflammation (Figure 1) (75). This evidence suggests a potential role of lactate transporter inhibitors in the therapy of RA.

#### 4. MCT/SMCT Inhibitors

Targeting lactate transporters has become a promising therapeutic avenue in oncology (76) and is also gaining attention in inflammatory disorders. A recent study found that blocking the lactate transporter MCT1 reduces the proliferation of breast cancer cells co-expressing MCT1 and MCT4 (77, 78) and reduces HIF-1 induced angiogenesis and tumour growth (20).

Several MCT inhibitors have been identified, although with only relative specificity for the various MCT isoforms. The first inhibitors identified were phloretin, flavonoids such as quercetin, stilbene disulphonates (including DIDS and 4,4'-dibenzamidostilbene-2,2'-disulphonate [DBDS]), and  $\alpha$ -cyano-4-hydroxycinnamate (CHC) and its analogues (32). Other inhibitors with a higher affinity for MCTs,

have been developed more recently (79). In this regard, AZD3965 is a dual MCT1 and MCT2 inhibitor, currently evaluated as an anticancer agent in Phase I clinical trials for patients with prostate cancer, gastric cancer or diffuse large B cell lymphoma (78, 80). Draoui et al. have recently identified several new compounds belonging to the 7-aminocarboxycoumarine family that potently inhibit MCTs (82).

Interestingly, 7-(N-benzyl-N-methylamino)- 2-oxo-2H-chromene-3-carboxylic acid (7ACC2) was further reported to be an inhibitor of lactate uptake that does not inhibit lactate export (82).

Samuvel et al. reported that alpha-cyano-4-hydroxycinnamic acid, an inhibitor of monocarboxylate transporters, blocks lactate-augmented inflammatory gene expression and NFκB activity in human macrophages, indicating that lactate transport through monocarboxylate transporters is required for macrophage effector functions (83). Besides MCTs, proton-sensing GPCRs such as T cell death-associated gene 8 (TDAG8) have been shown to be important for the modulation of T cells in an acidic tumour environment and during inflammation (84, 85).

Lonidamine is an anti-spermatogenic agent with anti-neoplastic activity; recently, it has been demonstrated that this drug inhibits lactate transport by MCT1, MCT2 and MCT4, providing an explanation to its anti-tumour effect (86, 87). Immunomodulatory drugs, such as thalidomide and its derivatives (lenalidomide and pomalidomide), have also been shown to act on the expression of MCT1 in myeloma cells, in vitro and in vivo: Eichner et al. demonstrated that these drugs destabilise the transmembrane complex CD147-MCT1, which is important in sustaining angiogenesis, proliferation and invasion of cancer cells, pointing out to the importance of lactate export and the efficacy of MCTs inhibition as an anti-cancer therapy (88).

Much less is known about SMCT inhibitors. Blocking SLC5A12 with a commercial antibody has revealed a great potential in promoting T cell egress from the inflamed site in a murine model of zymosan-induced peritonitis. More specifically, Phloretin (MCT1 inhibitor), an anti-Slc5a12 antibody or an isotype control antibody were injected intra-peritoneally in mice. Anti-Slc5a12 antibody caused

a significant reduction of CD4<sup>+</sup> T cells in the peritoneum in comparison to an isotype control antibody, while having no effect on CD8<sup>+</sup> T cells. In contrast, phloretin promoted a significant decrease of CD8<sup>+</sup> T cells in the peritoneum but did not show any effect on CD4<sup>+</sup> T cells. This suggests a peculiar specificity of these lactate transporter inhibitors targeting different T cells subsets (6).

Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and salicylic acid, and uricosuric drugs like probenecid, have also shown some inhibitor effects over MCT/SMCT substrates, such as hydroxybutyric acid (GHB), limiting their uptake (89, 90).

## 5. Expert opinion

For many years lactate has been considered a bystander product of glycolysis, produced during hypoxia or by highly proliferative cells. Only recently it has become to receive some consideration as a signalling metabolite. Its role in cancer biology and immunity is getting into the spotlight nowadays. The breakthrough that lactate plays an important role in the interplay between inflammation, cancer, metabolism and immunity together with the characterization of lactate transporters being expressed by a variety of cells (i.e. immune, stromal and cancer cells) has opened a new area of research and novel potential therapeutics. The emerging evidence that solid cancers deprive the tumour environment of glucose and enrich it with lactate which in turn depresses effector and cytotoxic T cell functions while promoting suppressive Treg cells, has also shed lights on new immunosuppressive therapy approaches, based on potentiating Treg cell activity, in conditions where Treg cell functionality is overthrown (i.e. autoimmunity, transplantation). On the other side, in cancer this can offer a new potential therapeutic approach based on targeting cancer metabolism that can reduce detrimental Treg cells in favour of effector T cells enhancing anti-tumour response (26) (Figure 1).

Different is the context of inflammatory disorders where the competition for nutrients between stromal and immune cells may be more even and lead to a different response to lactate.

Indeed, in the presence of high concentration of lactate, such as in the rheumatoid arthritis synovium, T cells are unable to egress ending up "entrapped" in the inflammatory site (6, 64, 65). Here, T cells produce a high amount of pro-inflammatory cytokines contributing to the establishment of chronic inflammation. Indeed, the effect of lactate on T cells recapitulates the typical characteristics of inflammatory infiltrates, in particular tissue retention, local production of inflammatory cytokines and loss of cytolytic activity (6) (Figure 1).

A selective lactate transporter expression by CD4<sup>+</sup> and CD8<sup>+</sup>-activated T cells may orchestrate their differential distribution in the inflamed tissues as well as affect their functional and migratory responses depending, for example, on the nature of the inflammatory exudate (i.e., more lactic acid versus sodium lactate) (91-93). Thus, modulating selective T cell subsets via targeting specific lactate transporters may provide novel tools to reduce inflammation and help to better understand the pathogenesis of inflammatory disorders.

The development of specific or pan-MCT/SMCT inhibitors capable of tissue specificity may be the ultimate goal to achieve targeted therapy. Little is currently known about the regulation of MCT/SMTCs expression and activity in different tissues and their regulation during inflammation or tumours. The generation of tissue/cell-specific MCT/SMTCs-deficient mice will enable exploiting MCT physiopathology and toxicity. MCTs/SMTCs substrate transport is another interesting area that should be further explored. Indeed, the discovery that MCTs can transport anticancer agents (i.e. 3-bromopyruvate, dichloroacetate and iodoacetate) across cell membranes (45) suggests that those substrates can also act as anticancer compounds. This can also lead to the identification and validation of biomarkers capable of predicting therapeutic responses.

Overall, we have provided a summary of the potential role of lactate that, via a distinctive signalling network, may promote pathogenic characteristic typical of the inflammatory or tumour "milieu". Thus targeting lactate transporters may provide a promising tool to reduce inflammation and induce anti-cancer responses.

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### **Reviewer disclosures**

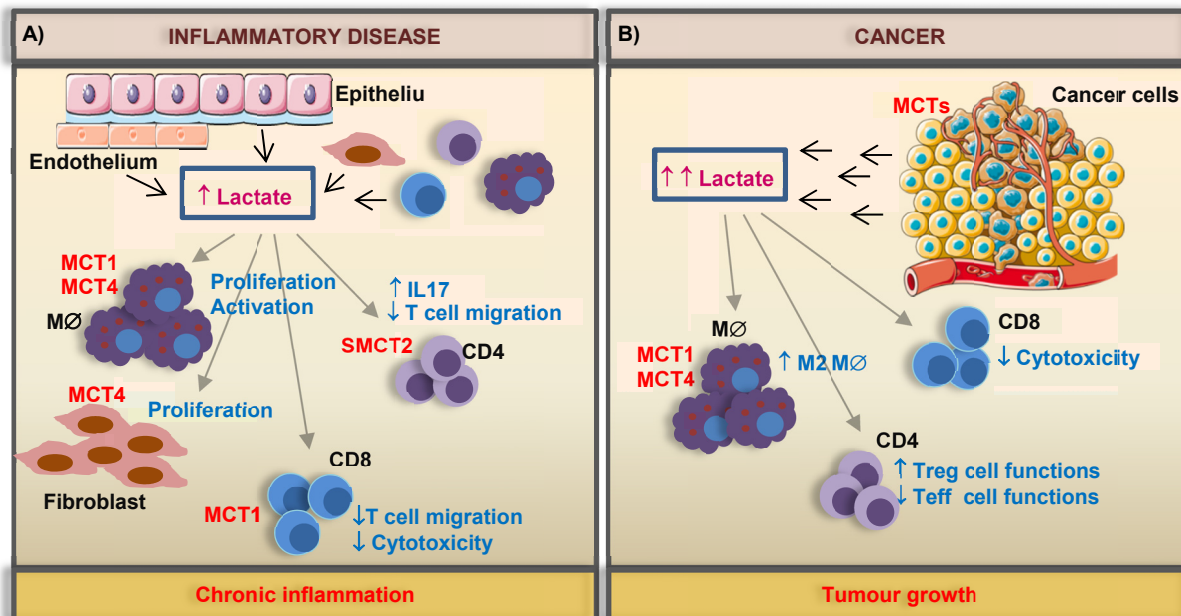
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**Table 1 - MCT transporters**

<b>MCT transporter</b>	<b>Common name</b>	<b>Substrate</b>	<b>Distribution</b>
SLC16A1	MCT1	Lactate, pyruvate, ketone bodies	Ubiquitous
SLC16A2	MCT8	T2, rT3, T3, T4	Ubiquitous
SLC16A3	MCT4	Lactate, ketone bodies	Skeletal muscle, chondrocytes, leucocytes, testis, lung, brain, ovary, placenta, heart, leucocytes
SLC16A4	MCT5	N/A	Brain, muscle, liver, kidney, lung, ovary, placenta, heart
SLC16A5	MCT6	Bumetanide probenecid nateglinide	Kidney, muscle, brain, heart, pancreas, prostate, lung, placenta
SLC16A6	MCT7	Ketone bodies	Liver, brain, pancreas, muscle, prostate
SLC16A7	MCT2	Pyruvate, lactate, ketone bodies	High expression in testis, moderate to low in spleen, heart, kidney, pancreas, skeletal muscle, brain and leucocytes
SLC16A8	MCT3	Lactate	Retinal pigment epithelium, choroid plexus
SLC16A9	MCT9	Carnitine	Endometrium, testis, ovary, breast, brain, kidney, spleen, retina
SLC16A10	MCT10	N/A	Kidney (basolateral), intestine, muscle, placenta, heart
SLC16A11	MCT11	N/A	Skin, lung, ovary, breast, lung, pancreas, retinal pigment epithelium, choroid plexus
SLC16A12	MCT12	N/A	Kidney, retina, lung testis
SLC16A13	MCT13	N/A	Breast, bone marrow stem cells
SLC16A14	MCT14	N/A	Brain, heart, muscle, ovary, prostate, breast, lung, pancreas liver, spleen, thymus

<b>HUGO nomenclature</b>	<b>Common name</b>	<b>Function</b>	<b>Distribution</b>
SLC5A1	SGLT1	Na <sup>+</sup> /glucose or Na <sup>+</sup> /galactose	Gastrointestinal tract, liver, kidney, male tissues
SLC5A2	SGLT2	Na <sup>+</sup> /glucose	Kidney, male tissues
SLC5A3	SMIT1	Na <sup>+</sup> /myoinositol	Ubiquitous
SLC5A4	SGLT3	Glucose-sensitive Na <sup>+</sup> -channel	Gastrointestinal tract
SLC5A5	NIS	Na <sup>+</sup> /iodide	Gastrointestinal tract, endocrine tissues, female tissues
SLC5A6	SMVT	Na <sup>+</sup> /biotin or Na <sup>+</sup> /pantothenate	Ubiquitous
SLC5A7	CHT1	Na <sup>+</sup> /Cl <sup>-</sup> /choline	Low expression in gastrointestinal tract, kidney, endocrine tissues, female and male tissues
SLC5A8	SMCT1	Na <sup>+</sup> /monocarboxylate	Intestin, kidney (apical membrane of tubular epithelial cells), brain, salivary gland, thyroid gland
SLC5A9	SMT	Na <sup>+</sup> /mannose	Gastrointestinal tract
SLC5A10	N/A	Unknown	Kidney
SLC5A11	SMIT2	Na <sup>+</sup> /myoinositol	Gastrointestinal tract, kidney, female tissues, brain
SLC5A12	SMCT2	Na <sup>+</sup> /monocarboxylate	Small intestine, kidney, brain, retina, male tissues, lymphoid organs, leucocytes

**Table 2 - SMCT transporters**



**Figure 1. Accumulation of lactate occurs in the inflamed tissue and in the tumour microenvironment where it promotes chronic inflammation and tumour growth.** A) During the inflammatory process immune cells sense (via specific transporters) and respond to the high levels of lactate that is produced by endothelial and epithelial cells as well as by the infiltrating immune cells, which all consume the available glucose. The sodium lactate transporter SMCT2 (SLC5A12) and the lactic acid transporter MCT1 (SLC16A1) are expressed by CD4<sup>+</sup> and CD8<sup>+</sup> T cells, respectively. MCT4 is expressed by fibroblasts and macrophages; the latter also display high levels of MCT1. Through these transporters, lactate modulates immune cell functions. In particular lactate promotes fibroblast proliferation as well as macrophage (MØ) proliferation and activation. Lactate also inhibits T cell motility thus causing T cell entrapment in the inflamed tissue. There, in response to lactate CD4<sup>+</sup> T cells produce higher amounts of IL-17 and CD8<sup>+</sup> cytotoxic activity is affected. Overall these events promote chronic inflammation. B) Cancer cells express high levels of MCTs and release high amount of lactate in the tumour microenvironment, as the product of their pronounced glycolytic metabolism, which leaves infiltrating immune cells reliant on carbon sources other than glucose for their metabolism. There, lactate has an effect on wide range immune cell functions. It induces the switch of macrophages to an M2 phenotype via the transporters MCT1 and MCT4. It also promotes T cell differentiation to the Treg phenotype while suppressing Teff functions (i.e. cytokine production, cytotoxicity), thus leading to enhanced tumour growth.



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